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Urine - Based Neisseria gonorrheae PCR Detection Kit Product Insert

Product # 30900

Neisseria gonorrhoeae is a Gram-negative coccus of the Neisseria genus. *N. gonorrhoeae* is usually seen in pairs infecting human cells. It has a circular DNA genome of approximately 1Mbp encoding over 2000 genes. *N. gonorrhoeae* is transmitted by sexual contact and usually causes infection in cells of the mucous membrane of the male urethra or the endocervix and urethra in females. During infection, polysaccharides are released from the bacteria that stimulate host cell production of tumour necrosis factors that cause an inflammatory response.

There is no vaccine against *N. gonorrhoeae* infection and antibiotic resistance is beginning to increase, therefore treatment includes a course of antibiotics that will be effective against resistant strains. Complications in males caused by the infection can result in prostatitis or orchitis if the bacteria spread. In females, invasion of the fallopian tubes or ovaries can result in salpingitis or ovaritis respectively, with any of these infections possibly resulting in sterility.

Principle of the Test

Norgen's Urine-Based *Neisseria gonorrhoeae* PCR Detection Kit constituents a ready-to-use system for the isolation and detection of *N. gonorrhoeae* using end-point PCR. The kit first allows for the isolation of total DNA, including bacterial DNA, from the urine samples using spin-column chromatography based on Norgen's proprietary resin. The bacterial DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for *N. gonorrhoeae* detection using the provided *N. gonorrhoeae* Master Mix. The *N. gonorrhoeae* Master Mix contains reagents and enzymes for the specific amplification of a 260 bp region of the *N. gonorrhoeae's* PorA gene. In addition, Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit contains a second Mastermix, the Control 2x PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided *Isolation Control (IsoC)*.. The amplification and detection of either the Isolation Control (IsoC) or the PCR control (PCRC) does not reduce the detection limit of the analytical *N. gonorrhoeae* PCR. The kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Solution A	10 mL
Solution B	15 mL
Wash Solution	9 mL
Elution Buffer	3 mL
Mini Filter Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
N. gonorrhoeae 2X PCR Master Mix	0.35 mL
Control 2X PCR Master Mix	0.35
Isolation Control (IsoC)*[a]	0.3 mL
N. gonorrhoeae Positive Control (PosC)*[b]	0.1 mL
Nuclease-Free Water	1.25 mL
Norgen's DNA Marker	0.1 mL
Product Insert	1

*IsoC = Isolateion Control; PosC= Positive Control
[a] The isolation control is a cloned PCR product
[b] The positive control is a fragment of N. gonorrhoeae cloned in a plasmid

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Lysozyme
- 96 – 100% ethanol
- 60°C incubator
- 15 mL tubes

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

The *N. gonorrhoeae* 2X PCR Master Mix, Control 2X PCR Master Mix, Isolation Control (IsoC), and *N. gonorrhoeae* Positive Control (PosC) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions

The user should exercise the following precautions while using the kit:

- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *N. gonorrhoeae* 2X PCR Master Mix, Control 2X PCR Master Mix, Isolation Control (IsoC) and *N. gonorrhoeae* Positive Control (PosC) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Urine-based *N. gonorrhoeae* PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

A. Specimen Collection, Storage and Transport

General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents.
- Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers

1. Specimen Collection and Sample Storage

- Midstream urine samples should be collected, as the first flow of urine has been shown to have a higher rate of contamination (Morimoto et al., 2003).
- It is highly recommended that urine samples be collected using Norgen's Urine Collection and Preservation Tubes (Cat# 18111). The urine samples can be stored for at least one year at room temperature when collected directly using Norgen's Urine Collection and Preservation Tubes.
- Alternatively, urine samples collected using any other collection and preservation systems or reagents are also compatible with this kit.

2. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material.

B. Isolation of DNA from Urine

Notes:

- Do not spin down or filter the urine sample before proceeding with the isolation, as this could negatively affect the isolation of N. gonorrhoeae DNA.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of **Wash Solution** by adding 21 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give you a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a 400 mg/mL stock solution (approximately 1.7 $x10^7$ units/mL) of lysozyme as per supplier's instructions.
- **Isolation Control (IsoC)**
    - An Isolation Control (IsoC) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) as indicated during the isolation procedure
    - The Isolation Control (IsoC) must not be added to the sample material directly.
    - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
    - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
- The PCR components of the Urine-Based *N. gonorrhoeae* PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

1. Add 300 µL of **Solution A** to 10 mL urine sample. Mix well by vortexing for 10 seconds. **(Note 1: Solution A must be mixed well before every pipetting**)
2. Centrifuge for **5 minutes at 2,000 RPM**, then discard the supernatant carefully in order not to dislodge the precipitated slurry pellet.
3. Add 20 µL of the previously prepared **lysozyme** to the precipitated slurry pellet. **Vortex for 10 seconds**. Incubate the mixture at **60°C for 20 minutes**
4. Add 500 µL **Solution B** to the precipitated slurry pellet, mix well by vortexing for 10 seconds.
5. Add 10 µL **Isolation Control (IsoC)** to the mixture from **Step 4.**
6. Add 500 µL of **96-100% Ethanol** to the mix from **Step 5**, mix well by vortexing for 10 seconds.
7. Transfer 650 µL from the previous mix into a Mini Filter Spin column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.
8. Repeat **Step 7** until the entire mixture from **Step 6** has been transferred to the Mini Filter Spin Column.
9. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute**. Discard the flowthrough and reassemble the spin column with its collection tube.
10. Repeat Step 9 to wash column second time.
11. Wash the column a third time by adding another 400 µL of **Wash Solution** to the column and centrifuge for **1 minute**. Discard the flow-through and reassemble the spin column with its collection tube.
12. Spin the column for **2 minutes** empty at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **1 minute at 14,000 RPM**.

C. *N. gonorrhoeae* PCR Assay Preparation

Notes:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of *N. gonorrhoeae* 2X Detection PCR Master Mix and Control 2X PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the *N. gonorrhoeae* 2X Detection PCR Master Mix and one PCR reaction using Control 2X PCR Master Mix should be set up in order to have a proper interpretation of the results.
- For every PCR run, one reaction containing *N. gonorrhoeae* Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of the *N. gonorrhoeae* Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using ***N. gonorrhoeae* 2X Detection PCR Mastermix**) and the PCR reaction for control detection (Set #2, using **Control 2X PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one *N. gonorrhoeae* detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. PCR Assay Preparation

PCR Components	Volume Per PCR Reaction
N. gonorrhoeae 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
Sample DNA	2.5 µL
Nuclease-Free Water	7.5 µL
Total Volume	20 µL

2. For each PCR set, prepare **one** positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

PCR Components	Volume Per PCR Reaction
N. gonorrhoeae 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
N. gonorrhoeae Positive Control (PosC)	10 µL
Total Volume	20 µL

3. For each PCR set, prepare **one** no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

PCR Components	Volume Per PCR Reaction
N. gonorrhoeae 2X PCR Master Mix OR Control 2X PCR Master Mix	10 µL
Nuclease-Free Water	10 µL
Total Volume	20 µL

D. *N. gonorrhoeae* PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one step PCR.

Table 4. *N. gonorrhoeae* Assay Program

PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 3	**Step 1**	**72°C**	**5 min**
Cycle 4	**Step 1**	**4°C**	**∞**

D. *N. gonorrhoeae* PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE 2% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The PCR products should be resolved on the 1X TAE, 2% Agarose gel at 150V for 30 minutes (Gel running time will vary depending on an electrophoresis apparatus).

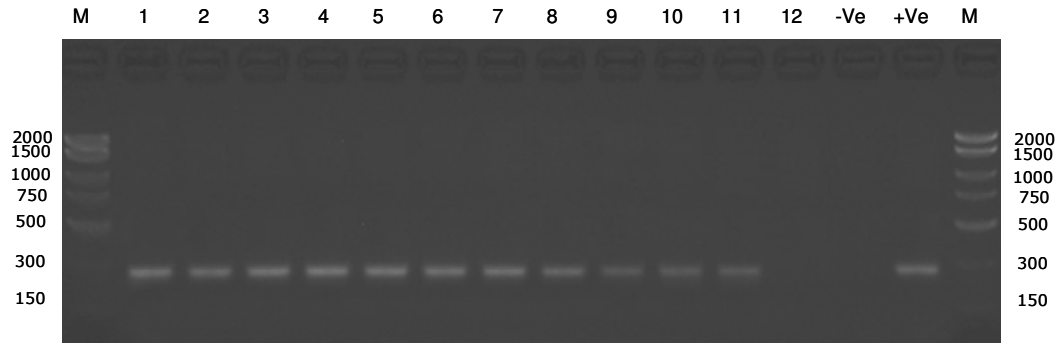

Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of *N. gonorrhoeae* at different concentrations (Target). The size of the *N. gonorrhoeae* target amplicon corresponds to the 260bp band represented by the provided DNA Marker (M). Lanes A-L represents samples spiked with different *N. gonorrhoeae* concentrations.

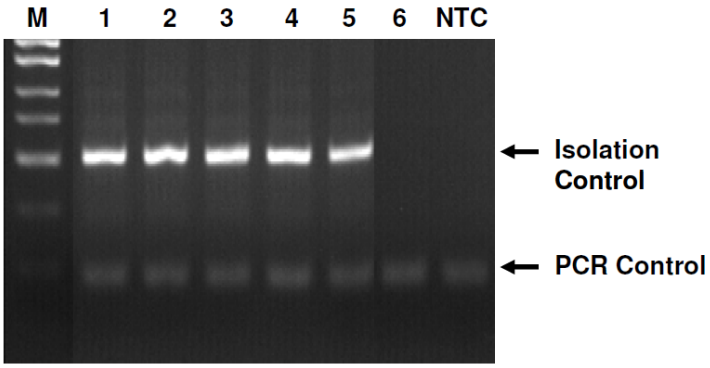

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the Control 2X PCR Master Mix. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. NTC=Negative Control.

Table 5. Interpretation of PCR Assay Results

Input Type	Target Reaction	Control Reaction		Interpretation
	***N. gonorrhoeae* Target Band (260 bp)**	**IsoC Band (499bp)**	**PCRC Band (150 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample		X		Negative
Sample			X	Re-Test
Sample				Re-Test
Sample	X	X		Positive
Sample	X		X	Positive
Sample	X			Re-Test

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

** Ignore any bands that appear between the Isolation Control band and the PCR Control band

E. Specificity

The specificity of Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit is first and foremost ensured by the selection of the *N. gonorrhoeae*-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses. Furthermore, the specificity of the *N. gonorrhoeae*-specific primers were tested against most of the known sexually-transmitted pathogens

F. Linear Range

- The linear range (analytical measurement) of Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit was determined by analyzing a dilution series of *N. gonorrhoeae* quantitative standard ranging from 8.46 x 10^9 copies/µl to 1 x 10^{-1} copies/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit has been determined to cover concentrations from 0.2 copies/µl to at least 8 x 10^6 copies/µl
- Under the conditions of Norgen's Urine DNA Isolation procedure, Norgen's Urine-Based *N. gonorrhoeae* PCR detection Kit covers a linear range from 200 copies/mL urine to at least 8 x 10^9 copies/mL urine.

G. Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's Urine-Based *N. gonorrhoeae* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a *N. gonorrhoeae* Positive Control (PosC) must be included. It is preferable to pool and test 6 samples at a time. If not, the provided *N. gonorrhoeae* Positive Control (PosC) is enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the *N. gonorrhoeae* PCR control nor the *N. gonorrhoeae* Isolation Control amplifies?

- If neither the *N. gonorrhoeae* PCR control (PCRC) nor the *N. gonorrhoeae* Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the positive control did not amplify the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the *N. gonorrhoeae* PCR control showed amplification but neither the *N. gonorrhoeae* targets nor the *N. gonorrhoeae* Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the *N. gonorrhoeae* Isolation Control was amplified in a sample?

- The sample tested can be considered as *N. gonorrhoeae* negative.

5. How should it be interpreted if only the *N. gonorrhoeae* targets and the *N. gonorrhoeae* PCR control were amplified in a sample?

- The sample tested can be considered as *N. gonorrhoeae* positive.

6. How should it be interpreted if only the *N. gonorrhoeae* targets was amplified in a sample?

- The sample tested can be considered positive. At high *N. gonorrhoeae* load, the *N. gonorrhoeae* amplicon will be predominant and the N. gonorrhoeae PCR control as well as the *N. gonorrhoeae* Isolation control may not amplify.

7. How should it be interpreted if only the *N. gonorrhoeae* PCR control and the *N. gonorrhoeae* Isolation control showed amplification?

- The sample tested can be considered negative

8. Can I process a different urine volume?

- The reagents provided with the isolation kit are only sufficient to process 24 urine samples of 5mL each.

9. What If I added more or less of the specified reagents' volume during DNA isolation?

- Adding less volume may reduce your DNA yields. Adding more may not affect the DNA yields EXCEPT if more Elution Buffer was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.

10. What If I forgot to do a dry spin after my second wash?

- Your DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your elution and it may interfere with your down stream applications.

11. What If I forgot to add the *N. gonorrhoeae* Isolation control during the Isolation?

The isolation must be repeated.

Related Products	Product #
Urine DNA Isolation Kit	18100
Urine DNA Isolation Kit for Exfoliated Cells or Bacteria	40750
Urine (Exfoliated Cell) RNA Purification Kit	22500
Urine Bacteria RNA Purification Kit	23400
Urine Protein Concentration Micro Kit	17400
Urine Protein Concentration Maxi Kit	21600

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Urine-based *N. gonorrhoeae* PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

References

Morimoto, M., Yanai, H., Chiba, H., Matsuno, K. and Shukuya, K. (2003). Importance of midstream clean-catch technique for urinalysis, reconfirmed by urinary flow cytometry. Clin Chim Acta. 333, 101-102.

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